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PRINCIPAL INVESTIGATOR: Graeme B. Bolger, M.D.

CONTRACTING ORGANIZATION: The University of Alabama at Birmingham
Birmingham, Alabama 35294

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Graeme B. Bolger, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Alabama at Birmingham
Birmingham, Alabama 35294

E-Mail: Graeme.Bolger@ccc.uab.edu

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13. ABSTRACT *(Maximum 200 Words)*

We have developed a novel approach to the cloning of tumor suppressor genes in prostate cancer. We have transferred large pieces of human DNA, cloned into bacterial artificial chromosomes (BACs), into human prostate cancer cell lines. We then tested the ability of the transferred human DNA to revert (render less tumorigenic) the neoplastic phenotype of the cancer cell lines, using several criteria, including morphological changes in the cells, doubling time and growth in soft agar. We have found several BACs that revert human prostate cancer cell lines in this assay. We are now testing the ability of cDNAs encoded by genes on these BACs to revert the cell lines, using identical assays. This process should allow us to identify the putative tumor suppressor gene on the BACs. Our approach is potentially applicable to the cloning of any human prostate tumor suppressor gene, and thus is of potentially major importance.

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INTRODUCTION

Cloning and study of tumor suppressor genes in prostate cancer has the potential to expand our knowledge of the pathogenesis of this disease and improve diagnosis, risk stratification, and therapy. We proposed a novel approach to the cloning of tumor suppressor genes in prostate cancer. As part of this plan, we developed methods for the transfer of large pieces of human DNA cloned into bacterial artificial chromosomes (BACs) into human prostate cancer cell lines. We then used several different assays to test the ability of the transferred human DNA to revert (render less tumorigenic) the neoplastic phenotype of the cancer cell lines. These assays included morphological changes in the cells, doubling time and growth in soft agar. The assays were used to determine if a particular BAC contains a gene that reverts the transformed phenotype of the cell line, which would be a putative human tumor suppressor gene. We identified several overlapping BACs, all of which are located in a single chromosomal region that has previously been implicated as containing a putative prostate cancer suppressor gene, that reproducibly revert several different human prostate cancer cell lines (PPC-1, PC-3) in these assays. We then identified several cDNAs encoded by genes located on our BAC contig and are planning to transfect these into the cell lines and test them for reversion. Using this strategy, we will be able to identify the tumor suppressor gene. Our approach is potentially applicable to the cloning of any human prostate tumor suppressor gene, and thus will be potentially of major importance.

BODY:

CLONING OF TUMOR SUPPRESSOR GENES IN PROSTATE CANCER BY A NOVEL TUMOR REVERSION METHOD.

Note: This Final Report is sub-divided into the various tasks in the revised and approved Statement of Work. The last approved Statement of Work was dated August 2000, as part of the transfer of the project with the move of the principal investigator (P.I.) from the University of Utah to the University of Alabama at Birmingham.

Task 1: To develop methods for the efficient and stable transfer of human DNA segments cloned in YACs or BACs into human prostate cancer cell lines (Months 1-18).

-- Target YACs with the neomycin-resistance vector and selected YACs with the GFP cassette (Months 1-6).

This portion of this task was successfully accomplished in the time scheduled. We obtained YACs from a variety of sources, including our then-colleagues at the University of Utah, and targeted them with standard YAC targeting vectors.

-- Test for the ability to introduce the YACs into prostate cancer cell lines using polyethylene glycol mediated fusion (Months 3-9).
-- Test for the ability to introduce the YACs into prostate cancer cell lines using lipid-mediated transfection (Months 3-9).
-- Test for the ability to introduce the YACs into prostate cancer cell lines using microinjection (Months 6-18).

These three portions of the task (which are all related) all ultimately proved impossible. Each of these technologies was tried repeatedly over an 18-month period. The methods tested were obtained from published literature (see references in initial application) that had been successful in introducing YACs into a variety of cell lines, most notably mouse embryonic stem (ES) cells. We concluded that these studies are much more difficult to perform in human prostate cancer cell lines, compared to ES cells and even other human cell lines. In addition, while we were performing these studies, data from several other groups, working on various aspects of the Human Genome Project, had demonstrated that YACs have an extremely high level of chimerism (1), which is a severe problem in performing functional studies with YACs generally.

Task 2: To isolate the human tumor suppressor gene located on a contig of YACs or BACs that produces phenotypic reversion of human prostate cancer cell lines (Months 18-42).

-- Transfer BACs into the cell lines by co-transfection, and obtain neomycin-resistant clones (Months 18-42).

This portion of this task was successfully accomplished with a few BACs prior to the submission of the original proposal (see initial proposal). We routinely used these methods after we had moved the University of Alabama at Birmingham. We reproducibly obtained 10 to 50 neomycin-resistant colonies per BAC per experiment.

-- Assay the clones for the presence of BAC DNA in the clones by PCR for the vector "arms," or by PCR for polymorphisms present on the BAC (Months 18-24).

We have developed PCR primers that amplify the vector arms of the BAC. These primers recognize the sequence of the chloramphenicol-resistance gene that is present in the BAC cloning vector. We have found that approximately 20-50% of the neomycin-resistant clones generated in a typical transfection (see previous paragraph) are positive on PCR for the vector. As a positive control for DNA quality in these experiments, we have used PCR primers that amplify any one of several human genes.

-- Test neomycin-resistant clones for cloning efficiency in soft agar and assess doubling time, and record morphology (Months 18-42).

We have successfully isolated and expanded clones from transfectants of at least 10 different BACs into two different human prostate cancer cell lines (PPC-1 and PC-3). All the BACs that we have tested are located in a single contig that overlaps the position of a putative human prostate tumor suppressor gene. The BACs were mapped to this region by the group at Washington University in St. Louis (2). All BACs were obtained from commercial sources (InCyte, St. Louis MO or Research Genetics, Huntsville AL). We have identified three BACs, all from a small region within the area of interest and all of which overlap each other, that produce morphological changes in the cells (i.e., flattened morphology), slow the growth of the cells and greatly reduce cloning efficiency in soft agar.

-- Fragment BACs, transfer them into cell lines, and assay for reversion (to determine the exact location of the gene on the BACs; [Months 18-42, depending on how quickly a reverting clone is obtained in the previous steps]).

We have modified this portion of the task for two reasons: (1) we have found that the BACs that appear to produce phenotypic reversion all partially overlap (i.e., they share a small common region). Therefore, we were able to localize the location of the putative tumor suppressor without having to physically fragment the BACs. (2) All the BACs that we are using have been completely sequenced by the Sanger Center or at Washington University in St. Louis. Their DNA sequences are available from the GenBank database. This means that we have already identified several candidate genes within the overlap region. We are in the process of obtaining cDNAs for these genes. It is possible that there are additional genes on the BACs that we have missed. However, it seemed reasonable to us to test the known genes as cDNAs first, before attempting to look for hitherto-unidentified genes on the BACs.

KEY RESEARCH ACCOMPLISHMENTS:

-- We have obtained data that strongly support our original hypothesis, in that we have been able to introduce BACs into human prostate cancer cell lines and then able to assay the genes on the BAC for their ability to produce phenotypic reversion of the cells.

-- Further experiments, not included in the original Statement of Work, should be able to confirm the hypothesis. In particular, we need to isolate the cDNA encoded by the gene of interest and demonstrate that it can produce phenotypic reversion of the cell lines. We can then use other functional and mutational analysis to verify that the cDNA indeed encodes for a tumor suppressor.

REPORTABLE OUTCOMES:

No reportable outcomes have been generated to date. We believe that successful completion of at least some of the additional experiments described in the preceding paragraph will be necessary for our data to be completely convincing.

CONCLUSIONS:

If our data can be confirmed by the additional studies outlined in KEY RESEARCH ACCOMPLISHMENTS, we believe that we will have reached two important objectives: First, we will have cloned a novel human prostate cancer suppressor gene. Further study of the biological and clinical significance of this gene and its protein should provide important insights into the biology of prostate cancer generally. Secondly, we will have demonstrated the feasibility of our overall approach to the identification of human prostate cancer tumor suppressor genes. As our approach is potentially applicable to the cloning of any human prostate tumor suppressor gene (and also to the cloning of tumor suppressor genes in other human cancers), it is of potentially major importance.

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(none)

PERSONNEL:

Graeme B. Bolger, M.D., P.I.
Aileen Olsen, Research Technician, (University of Utah)
Lisa High-Mitchell, Research Assistant, (University of Alabama at Birmingham)

APPENDICES:

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